Eur. J. Soil Biol., 1993, **29** (1), 13-16

Effects of tannin compounds on two species of Collembola

N. Poinsot-Balaguer (1), L. Racon (2), N. Sadaka (3), and J. Le Petit (2)

(1) *Laboratoire de Biosystématique et Ecologie Méditerranéenne,*
Université de Provence, UA 1152, Centre Saint-Jérôme,
13397 Marseille, Cedex 20, France
(2) *Laboratoire de Microbiologie.*
Faculté des Sciences et Techniques de Saint-Jérôme,
13397 Marseille, Cedex 20, France
(3) *Laboratoire d'Ecologie*
Animale Université Cadi-Ayyad, Marrackech, Maroc.

Received August 5, 1991; accepted July 10, 1992.

Abstract

The effects of condensed and hydrolysable tannin extracted from evergreen oak leaves (*Quercus ilex* L.) were tested on two species of Collembola. Concentrations lower than those present in the litter leaves were toxic to these insects. Detoxification by washing with rainwater and biodegradation of tannic compounds are necessary for litter decomposition, in which microarthropods participated, to occur.

Keywords: tannins, evergreen oak, Collembola.

Effets de composés tanniques sur deux espèces de Collemboles.

Résumé

Les effets de tanins condensés et hydrolysables, extraits de feuilles de chêne vert (*Quercus ilex* L.), ont été testés sur deux espèces de Collemboles : *Onychiurus zschokkei* Handschin et *Folsomia candida* Willem. Des concentrations, plus basses que celles mesurées dans les feuilles de la litière, sont toxiques pour les Collemboles. La mort survient le premier jour de l'expérience. La détoxification par les eaux de pluies est nécessaire pour que les microarthropodes participent à la biodégradation de la litière.

Mots-clés : tanins, chêne vert, Collemboles.

INTRODUCTION

Soil invertebrates are responsible for 10% or less of the respiratory metabolism of most ecosystems (Petersen and Luxton, 1982). Plant decomposition depends on several factors including the chemical structure of the organic matter present and its appeal to the microfauna capable of eating it. The plant chemistry is probably the most fundamental factor which determines whether a plant is eaten at all, and how much is eaten once feeding begins (Bernays and Chapman, 1978).

Plant tannins are capable of precipitating proteins as well as digestive enzymes (Hagerman and Butler, 1980). They can therefore impede digestion and cause poisoning or reduce the available choice or organic plant matter to the insects (Robbins *et al.*, 1987). Insects' response to tannins differ because some degrees of counteradaptation are possible in coevolved species. Tannins may thus possess toxic effects other than a reduction of digestibility (Berenbaum, 1983).

In a previous study (Sadaka and Poinsot-Balaguer, 1987; 1989), two species of Collembola *Onychiurus zschokkei* Handschin 1919 and *Folsomia candida* Willem 1902, were fed with evergreen oak (*Quercus ilex* L.) leaves harvested in the canopy and litter. Four stages of leaves were considered:

- 1-year-old.

- 2-year-old.
- Leaf typical of the litter upper layer (L^1).
- Leaf still recognizable in spite of decay constituting the litter underlayer (L^2).

These leaves contained some tannin cells within the sclerenchyma and the lower epidermis but the tanin was mainly concentrated in the upper epidermis (Racon *et al.*, 1988). The tannins present belonged to different phenolic groups: hydrolysable tannins composed of gallic acid or elligic esterified to a sugar moiety and condensed tannins which are polymers derived from flavan-3-ols.

The toxicity of the tannins extracted from evergreen oak leaves was tested on the two species of Collembola specified above.

MATERIAL AND METHODS

Two Collembola species were tested: *Onychiurus zschokkei* and *Folsomia candida*. The first species was collected in the litter of evergreen oaks growing in dense copses planted in La Gardiole de Rians (Var, France) The copses were 50 years old and formed a continuous cover. The station climate and vegetation have been described previously (Barbero and Loisel, 1983). The second, *Folsomia candida,* reared in the Museum of Natural History, is parthenogenic and has a particularly high reproduction rate.

O. zschokkei and *F. candida* were reared in Petri dishes (5 cm in diameter) with a fiberglass filter disc (Whatman) on the bottom. This disc was soaked with a nutritive solution composed of 1% glucose and 0.2% "Difco" yeast extract. To this nutritive solution, which was used as the baseline solution, was added 1.5 ml of a solution of condensed or hydrolyzable tannins at various concentrations in each Petri dish. The tannin concentrations were selected above and below the maximum concentration measured in the leaves.

The tannins used in this study were extracted from 2-year-old leaves, which contained the highest tannin concentrations (Racon *et al.*, 1988): the hydrolysable tannins amounted to 0.537% and the condensed tannins to 2.144% of the dry matter. The leaves were washed, dried, and then treated according to the method of Broadhurst and Jones (1978) to separate tannins from other plant compounds. Hydrolysable tannins were separated from condensed tannins by ethyl acetate extraction. Hydrolysable and condensed tannins were then purified separately, using the chromatography method described by Hagerman and Butler (1980) on a Sephalex LH 20 column (30 × 2 cm). The flow of the eluant was maintained at 0.8 ml min^{-1} by means of an Analtrap EC 93-TM pump. The eluted compounds were detected with a Pye Unicam ultraviolet visible detector. An initial elution with ethanol sufficed to remove the proteins and other contaminants. The tannins were then eluted with dioxane-water (45:55, v/v) with detection at 540 nm. At this wavelength the specific extinction coefficients of tannins are low (E=0.12 in the case of condensed tannins, E=0.072 in that of hydrolyzable tannins), but there is no interference from contaminating compounds.

To the nutritive solution used as the control solution, were added various volumes of distilled water plus tannin solutions.

The experiment was conducted at 20°C. The test was carried out on 10 individuals with each solution and each tannin category and replicated three times.

The mortality was expressed as a percentage, after a ten day experimental period.

RESULTS AND DISCUSSION

At all the tannin concentrations tested all the deaths occured during the first day of the experiment. The insects that survived outlasted the duration of the experiment. The concentration scales corresponding to the Collembola sensitivity differed between the hydrolysable and condensed tannins. The mortality rate was proportional to the tannin concentration (*fig.* 1 and 2).

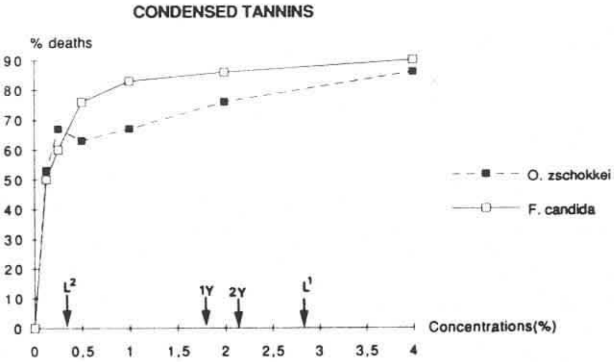

Figure 1. – Effects of condensed tannins at various concentrations on the two species of Collembola, *Onychiurus zschokkei* Handschin and *Folsomia candida* Willem.

1 y=1-year-old leaves on the tree.

2 y=2-year-old leaves on the tree.

L^1=whole leaves typical of the litter upper layer.

L^2=typical leaves still recognizable in spite of decay, constituting the litter underlayer.

Covariance analysis indicated that percentage of deaths within the two species varied significantly with condensed and hydrolysable tannins concentrations (table 1).

A comparison of the overall sensitivity of *Onychiurus zschokkei* and *Folsomia candida* to various concentrations of the groups of tannins showed no significant difference (test U Mann Whitney).

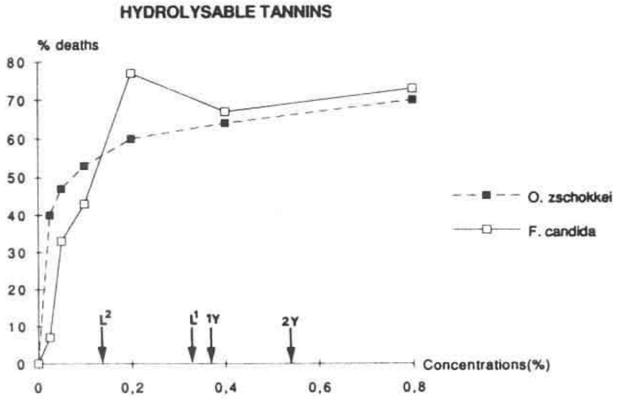

Figure 2. – Effects of hydrolysable tannins at various concentrations on the two species of Collembola *Onychiurus zschokkei* Handschin and *Folsomia candida* Willem.

1 y=1-year-old leaves on the tree.

2 y=2-year-old leaves on the tree.

L^1=whole leaves typical of the litter upper layer.

L^2=leaves typical still recognizable in spite of decay, constituting the litter underlayer.

Table 1. – Analysis of responses of *Onychiurus zschokkei* and *Folsomia candida* to six condensed and hydrolysable tannin concentrations.

Species	*Onychiurus zschokkei*		*Folsomia candida*	
	Cond. Tannins	Hydr. Tannins	Cond. Tannins	Hydr. Tannins
Sum of squares	621.07	1615.29	669.91	2276.46
Mean square	621.07	1615.29	669.91	2276.46
F ratio	31.74	7.42	4.73	11.89
P	0.004	0.05	0.09	0.02

A comparison of the responses of *Onychiurus zschokkei* at two condensed tannin concentrations (0.125% and 0.25%) and its response at the two close hydrolysable tannin concentrations (0.1% and 0.2%) showed a significant difference (test U p=0.003). At these concentrations, hydrolysable tannins are more toxic than condensed tannins for this species, but a similar comparison for *F. candida* showed no significant difference. For the same concentrations there was no significant difference between the two species.

Comparisons between the experimental tannin concentrations and those measured within the leaves showed that only the L^2 stage leaves contained weaker concentrations than those corresponding to the maximum mortality rate.

In addition, over the ten day experimental period, red-brown spots appeared on the bodies and antennae of most of the individuals raised in the presence of tannin solution. The nature of these spots was not defined.

The experiments showed that tannins are toxic, even at weak concentrations. The results corroborated earlier observations (Sadaka and Poinsot-Balaguer, 1987; 1989; Sadaka *et al.*, 1988) concerning the feeding activity of the Collembola species *Onychiurus zschokkei* and *Folsomia candida*, when reared on functional evergreen oak leaves or on litter leaves at four stages of decomposition. The consumption of litter leaves was significantly higher than that of functional leaves. The longer the litter leaves had been lying on the ground, the higher their consumption rate.

The low palatability of functional leaves for *Onychiurus zschokkei* might be due to the presence of the tannin compounds. The tannin compounds in L^2 stage leaves undergo enzymatic hydrolysis or are released into the surrounding environment (Racon *et al.*, 1988), thus making the leaves palatable for Collembola. These insects have similar feeding behaviour to that of soil invertebrates. For example, soil invertebrates were more numerous in a beech litter which poor in tannin compounds (brown leaves with 0.15% of tannin) than in beech litter high in tannin compounds (yellow leaves with 0.55% of tannin). The thecamebians were 7 times more numerous, the rotifers 4 times more, mites 1,5 time more, the collembola 2.2 times more and the genus *Onychiurus* 5 times more numerous in the former than in the latter case (Kuhnelt, 1963).

According to Satchell and Lowe (1967), the litter's palatability increases along with its decomposition and the microbial degradation of tannins. In the less fully decomposed litter, a high tannin content can cause a reduction in the mineralization and availability of nutrients (Baldwin *et al.*, 1983).

Our data agree with those by Beck *et al.* (1969), on the litters of deciduous species. Leaves progressively lose their tannin compounds due to washing by rainwater or biodegradation on the soil surface and thus also lose inhibitory effects on living organisms. In the case of evergreen oak litter which is particularly rich in tannins, a decrease in these compounds' concentrations appears to be a necessary prerequisite for the survival of microarthropods, and for their intervention in the biodegradation phenomenon.

REFERENCES

Baldwin I. T., Olson R. K. and Reiner W. A. (1983). – Protein binding phenolic and the inhibition of nitrification in sub-alpine balsam fir soils. *Soil Biol. Biochem.*, **15**, 419-423.

Barbero and Loisel R. (1983). – Les chênaies vertes du Sud-Est de la France méditerranéenne. Valeurs phytosociologiques, dynamique et potentielle. *Phytocoenologia*, **2**, 225-244.

Berenbaum M. R. (1983). – Effects of tannins on growth and digestion in two species of Papilionids. *Entomology experimenta and applicata*, **34**, 245-250

Bernays E. A., Chapman R. F. (1978). – Plant Chemistry and Acridoid Feeding Behaviour. *In: Biochemical Aspects* *of Plant and Animal Coevolution*. Harbone JB (ed), Academic Press, New York, 99-137.

Beck G., Domergue Y. and Van der Driessche R. (1969). – L'effet litière. II. Etude expérimentale du pouvoir inhibiteur des composés hydrosolubles des feuilles et des litières forestières vis-à-vis de la microflore tellurique. *Oecologia Plantarum*, **4**, 237-266.

Broadhurst R. B. and Jones W. T. (1978). – Analysis of condensed tannins using acidified vanillin. *J. Sciences Food Chemistry and Agriculture*, **29**, 944-947.

Hagerman A. E. and Butler L. G. (1980). – Determination of protein in tannin-protein precipitates. *J. Agriculture Food Chemistry*, **28**, 944-947.

Hagerman A. E. and Butler L. G. (1980). – Determination of protein in tannin complexes for tannin analysis and plant defense mechanisms. *J. Chemical Ecology*, **13,** 1243-1259.

Kühnelt W. (1963). – Uber die Einfluss den Mycels von Clitocybe infundibuliformis auf die Streufauna. *In: Soils Organisms* Doeksen J.: Drift, J. Van der ed. North Holland, Amsterdam, 281-288.

Petersen H. and Luxton M. (1982). – A comparative analysis of soil fauna populations and their role in decomposition processes. *In*: Petersen H., ed. Quantitative ecology of microfungi and animals in soil and litter. *Oikos*, **39**, 287-388.

Racon L., Sadaka N., Gill G., Le Petit J., Matheron R., Poinsot-Balaguer N., Sigoillot J. C. and Woltz P. (1988). – Evolution of tannic compounds in evergreen oak leaves. Histological and chemical analysis. *Can. J. Botany*, **66**, 663-667.

Robbins C. T., Hanley T. A., Hagerdan A. E., Jelford O., Baker D. L., Schwartz C. C. and Hantz W. W. (1987). – Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology* **68**, 98-107.

Rosenthal G. A. (1986). – The chemical defenses of higher plants. *Scientific American*, **254**, 1, 76-82.

Sadaka N. (1986). – *Contribution à l'étude des relations trophiques collemboles – feuilles de chêne vert – micro-organismes*. Thèse Ecologie, Université Aix-Marseille I, France 133 p.

Sadaka N. and Poinsot-Balaguer N. (1987). – Relations trophiques entre le collembole *Onychiurus zschokkei* Handschin et les feuilles de chêne vert *Quercus ilex* L., à divers stades de décomposition. *Rev. Ecol. Biol. Sol.*, **24**, 329-340.

Sadaka N and Poinsot-Balaguer N. (1987). – Determination of aminoacids in leaves of evergreen oak at four different stages of decomposition. *Biol. Fert. Soils*, **5**, 158-163.

Sadaka N. and Poinsot-Balaguer N. (1989). – Relations trophiques feuilles de chêne vert (*Quercus ilex* L.) – Collemboles. Influence de la qualité du matériel foliaire sur la croissance pondérale d'*Qnychiurus zschokkei* Handschin. *Rev. Ecol. Biol. Sol*, **2**, 197-204.

Sadaka N., Poinsot-Balaguer N. and Talin J. (1988). – Relations trophiques feuilles de chêne vert (*Quercus ilex* L.) – Collemboles. Influence de la qualité du matériel foliaire sur la biologie d'*Onychiurus zschokkei* et *Folsomia candida* Willem. *Vie et milieu*, **39**, 1, 33-39.

Satchell J. E. and Lowe D. G. (1967). – Selection of leaf litter by *Lumbricus terrestris*. *In: Progress in Soil Biology*, Graff O., Satchell J. E. (ed), North Holland, Amsterdam, 102-119.